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SELECTIVE INTRACOMPLEX REACTION OF PENDANT GROUPS IN A PURINE-PYRIMIDINES BASE PAIR

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Abstract: Evidence is presented suggesting that hydrogen-bonded base pairing and/or aryl stacking interactions between derivatives of guanine (9-[(4-aminobutyloxy)-methyl]guanine) and cytosine (1-([(2-benzyloxy)ethoxy]methyl)cytosine) lead to enhanced intracomplex chemical reaction between the corresponding amino and ester groups. On the basis of analysis of the kinetic data it is concluded that a chain length of four methylene groups (4-amino- butyloxy as compared to 2-aminoethoxy or 6-aminohexyloxy) on the guanine is necessary to achieve the appropriate geometry for intracomplex reaction. Support for the reaction scheme is provided by the absence of reaction between the guanine derivative and a corresponding ester derivative of thymine, not expected to associate.

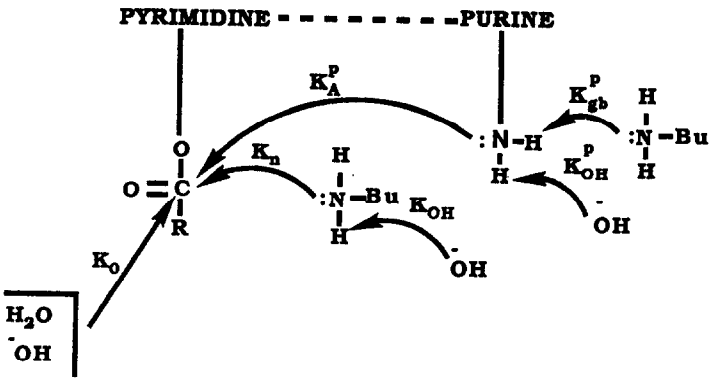
INTRODUCTION

One important feature affecting enzymatic reaction efficiency is believed to be the specifity of binding and precise orientation of the reacting species in the enzyme substrate complex. Based in part upon this view, the last two decades have seen the design of many synthetic models involving intramolecular reactions of covalently bound reactants[1] and bimolecular reactions resulting from specific binding interactions (electrostatic, hydrophobic) of reactants.[2,3,4] Host and guest molecular recognition has also attracted much interest and selective hosts for anionic[5] cationic[6] and hydrophobic[3a,7] guests have been synthesized.

The base pairing of purines and pyrimidines[8a,8b] which is essential for the intramolecular attraction and recognition in nucleic acids would appear attractive as a model for bringing together two different potentially reactive groups for selective chemical reaction. Several approaches to the design of such model systems have recently been reported in the literature.[8,9]

Here we report that hydrogen bonded base pairing and/or aryl stacking interactions between a guanine linked to an alkylamine and a cytosine linked to an alkylbenzoate lead to enhanced intra-complex reaction. The kinetic model in the presence of n-butylamine as buffer and an external catalyst is depicted in Scheme 1.

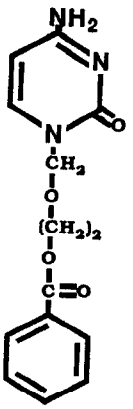
scheme 1



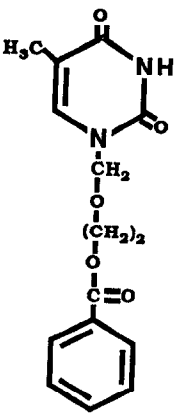
The dotted line denotes purine-pyrimidine coupled bases linked to an amino group and ester moiety, respectively. $BuNH_2$ and OH are external catalysts.

k_n and k_{OH} represent rate constants of nucleophilic attack by external amines and specific base catalyzed external amine attack on the ester site in the purine-pyrimidine pair, respectively. k^P_{gb} and k^P_{OH} however, denote the $BuNH_2$ and ^-OH catalyzed nucleophilic attack of Purine-NH_2 in the ternary activated complex and k^P_A corresponds to the internal, uncatalyzed nucleophilic attack of Purine-NH_2. k_o includes the water and specific base rate constant.

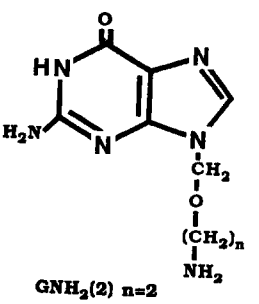
The following esters and catalysts were prepared:



C-Benz



T-Benz



GNH_2(2) n=2
GNH_2(4) n=4
GNH_2(6) n=6

EXPERIMENTAL

The syntheses of C-Benz, T-Benz and GNH_2 (n=2,4,6) were performed by the chloromethyl alkylation according to described procedures[10,11,12] and had physical properties in accord with the structures given.

1-[[2-(Benzoyloxy)ethoxy]methyl]cytosine, (C-Benz)

m.p.=185°C, 187-188°C lit[9], ^{1}H-nmr: δ3.7, 9.17 (m,4H(CH_2CH_2), δ 5.02 (s,2H, (OCH_2N)), δ 5.88 (d, 1H (C5)), δ 7.02, 7.63 (m, 8H (NH_2, C6, A_r)).

M.S.: 289M^+, 179, 141, 111, 105.

Anal. calc'd. for $C_{14}H_{15}N_3O_4$; C, 58.1; H, 5.25; N, 14.53; Found: C, 58.17; H, 5.41; N, 14.72.

1-[[2-(Benzoyloxy)ethoxy]methyl] thymine, (T-Benz)

m.p.=115°C, 115-116°C lit[9], ^{1}H-nmr: δ1.85, (s,3H(CH_3)) δ 3.9, 4.7 (dt, 4H (CH_2CH_2) δ 5.2 (s, 2H (OCH_2N) δ 7.1-7.43 (m, 7H, (C(3), C(6), Ar).

M.S.: 304M^+, 227, 199, 184, 170, 155, 139, 125.

Anal. calc'd. for $C_{15}H_{16}N_2O_5$; C, 59.21; H, 5.3; N, 19.72; H,5.25; N, 9.47.

Found: C, 59.67; H, 5.25; N, 9.74.

All the 9-[aminoalkyloxy]methyl derivatives of guanine were prepared as their phthaloyl analogs. The phthaloyl protective groups were cleaved with hydrazine followed by 1M HCl.

9-[(2-Aminoethoxy) methyl]guanine.HCl (GNH_2(2)).

m.p. = 228-230°C; ^{1}H-nmr (d^6-DMSO); δ 7.84 (s, 1H (C8)), δ 6.95 (br.s, 2H (Purine NH_2), δ 5.4 (s, 2H (NCH_2O)), δ 3.55 (t, 2H (OCH_2) δ 2.84 (t, 2H (CH_2N)).

M.S.: 225M^+, 224, 208, 194, 180, 164, 150.

Analysis calc'd for the phtalimido derivative $C_{16}H_{14}N_6O_4$, C,54.23; H, 3.95; N, 23.72; Found: C, 54.82; H, 3.8; N, 23.4.

9-[(4-aminobutyloxy)methyl]guanine.HCl (GNH_2(4).

m.p. = 246°C; ^{1}H-nmr (d^6-DMSO): δ 7.85 (s,1H (C8)), δ 6.94 (br,s, 2H (Purine NH_2), δ 5.4 (s, 2H (NCH_2O)), δ 3.81, 3.6 (m, 6H ($CH_2CH_2CH_2$)) δ 2.87 (t, 2H (CH_2N)).

M.S.: 254M^+, 253, 239, 225, 211, 180, 164, 150.

Anal. calc'd for the phthalimido derivative $C_{18}H_{18}N_6O_4$. C,56.54; H,4.71; N,21.98; Found: C,56.74; H,4.65; N,21.59.

9-[(6-aminohexyloxy)methyl]guanine.HCl (GNH_2(6).

m.p. = 250°C; ^{1}H-nmr (d^6-DMSO): δ 7.85 (s,1H (C8)), δ 6.95 (br,s, 2H (Purine NH_2), δ 5.4 (s, 2H (NCH_2O)), 3.95-3.6 (m, 12H), 1.6(s,2H(aliphatic NH_2)).

Anal. calc'd for the phthalimido derivative $C_{20}H_{22}N_6O_4$. C,58.53; H,5.36; N,20.48; Found: C,58.72; H,5.21; N,20.22.

The kinetic measurements of C-Benz hydrolysis were carried out spectrophotometrically by monitoring the change in absorbance V_s time at 234nm in the presence of external buffer (catalyst): butylamine (0-0.6M) or K_2CO_3(0-0.1M) at pH 10.34, T=35.4°C.

The concentrations of C-Benz and of GNH_2 in solution were both $1.1x10^{-3}$M.

RESULTS AND DISCUSSION

The rate expression in the presence of butylamine as external catalyst and buffer is given in equation 1.

$k_{obs}=k_o+k_n[BuNH_2]+k_{OH}[^-OH][BuNH_2]+k^P_{gb}[BuNH_2]+k^P_{OH}[^-OH]+k^P_A$.

Since the values of the rate constants k_o, k_n and k_{OH} of the coupled C-Benz-GNH_2 ester are assumed to be close to the corresponding rates resulting from the isolated C-benz ester, the difference between the slopes of the two systems and intercepts of the two systems determined by the linear plot of k_{obs} $V_o[BuNH_2]$, should equal k^P_{gb} and $k^P_{OH}[^-OH]$ + k^P_A respectively.

With K_2CO_3 as buffer the observed rate constant obey equation 2:

$$k_{obs} = k_{hyd} + k^P_{OH}[OH] + k^P_A + k_c[CO^{--}_3] \qquad (2)$$

Here again the difference between the intercepts of the coupled and uncoupled esters determined by plots of k_{obs} $V_o[CO_3^{--}]$ equal to $k^P_{OH}[OH]$ + k^P_A.

Fig 1 exhibits the dependence of C-Benz on $BuNH_2$ in the presence and absence of GNH_2 (n=4) at pH=10.3 T=35.4°C. The slopes and intercept values of C-Benz in the presence of $BuNH_2$ or K_2CO_3 with and without the complementary base GNH_2 (n=2,4,6) are assembled in Table 1. The data of T-Benz in aquous solution and of C-Benz in dioxane solution is also included.

From Table 1 it is inferred that the spatial arangement of the nucleophilic amino group attached to the complementary base, relative to the ester carbonyl group is crucial for catalysis. Only chain length n=4 on GNH_2 is appropriate for catalysis; no catalysis was observed with GNH_2 when n=2 or 6.

The calculated rate constants for n=4 are: $k^P_{gb}=8.10^{-3}M^{-1}$ min^{-1} and $k^P_{OH}[OH]$ + $k^P_A=3.1x10^{-3}$ min^{-1} (Table 1, entry 1,2 and entry 3,4). Additional evidence for our model involving a specific oriented disposition requirements are: a) addition of 5M urea abolished catalysis, b) substrates which are not expected to form base pairs (T-Benz + GNH_2 (n=4)) do not exhibit internal catalysis (Table 1 entries 7,8).

In aqueous solution it seems most likely that base association[13] involves a stacking arrangement. In organic solvents however base pairing due to hydrogen bonding probably takes place. We have therefore also examined the catalytic effect by GNH_2 in dioxane as a solvent (Table 1, entry 9,10).

In non-aqueous solvent the kinetic terms contributing to catalysis according to equation 1 are: K_o, k_n, k^P_{gb} and k^P_A.

From Table 1 it is inferred that C-Benz + $BuNH_2$ in the absence of the complementary base (GNH_2) does not display any substantial catalysis (k_n=0) however in the presence of GNH_2 (n=4), k^P_{gb} and k^P_A contribute significantly and have values of $2.4x10^{-3}M^{-1}$ min^{-1} and $2.8x10^{-4}$ min^{-1} respectively.

In conclusion, our results indicate that reaction selectivity may be brought about by purine-pyrimidine base stacking and or base pairing. Further studies involving more efficient recognition sites and examining additional reactions, in modified purine-pyrimidine bases are now called for.

Table 1
Slopes and intercepts calculated by equations 1 and 2 for C-Benz and T-Benz with and without the complementary base GNH_2 (n=2,4,6) pH=10.3, T=35.4°C.

No.	Ester	amine	Buffer	Slope M^{-1} min^{-1}	intercept min^{-1}
1	C-Benz	$GNH_2(4)$	$BuNH_2$	13.2×10^{-3}	5.2×10^{-3}
2	C-Benz	-	$BuNH_2$	5.2×10^{-3}	2.1×10^{-3}
3	C-Benz	$GNH_2(4)$	K_2CO_3	1.35×10^{-3}	5.1×10^{-3}
4	C-Benz	-	K_2CO_3	1.42×10^{-3}	2.1×10^{-3}
5	C-Benz	$GNH_2(2)$	$BuNH_2$	2.36×10^{-3}	1.75×10^{-3}
6	C-Benz	$GNH_2(6)$	$BuNH_2$	3.1×10^{-3}	2.0×10^{-3}
7	T-Benz	-	$BuNH_2$	1.7×10^{-3}	1.75×10^{-3}
8	T-Benz	$GNH_2(4)$	$BuNH_2$	2.0×10^{-3}	1.83×10^{-3}
9	C-Benz*	$GNH_2(4)$	$BuNH_2$	2.4×10^{-3}	0.4×10^{-3}
10	C-Benz*	-	$BuNH_2$	0.0	0.12×10^{-3}

* in dioxane.

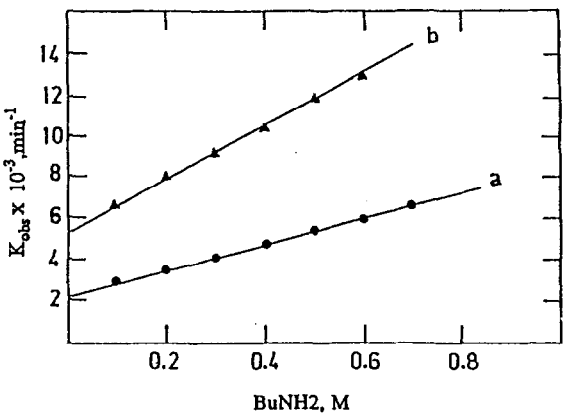


Fig. 1. First order rate constants of C-Benz V_s $BuNH_2$ concentration:
a) Without adding $GNH_2(4)$ (-●-●-).
b) With the adding of $GNH_2(4)$ (-▲-▲-), pH=10.3, T=35.4°C.

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